DOT/FAA/AM-98/5

Office of Aviation Medicine
Washington, D.C. 20591

Selection of an Internal Standard for Postmortem Ethanol Analysis

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February 1998

Final Report

U.S. Department of Transportation
Federal Aviation Administration

NOTICE

Technical Report Documentation Page

1. Report No.	2. Government Accession No.	3. Recipient's Catalog No.
DOT/FAA/AM-98/5		

4. Title and Subtitle	5. Report Date
Selection of an Internal Standard for Postmortem Ethanol Analysis	February 1998
	6. Performing Organization Code

7. Author(s)	8. Performing Organization Report No.
Canfield, D.V., Smith, M.D., Adams, H.J., and Houston, E.R.	

9. Performing Organization Name and Address	10. Work Unit No. (TRAIS)
FAA Civil Aeromedical Institute P.O. Box 25082, Oklahoma City, OK 73125	11. Contract or Grant No.

12. Sponsoring Agency name and Address	13. Type of Report and Period Covered
Office of Aviation Medicine Federal Aviation Administration 800 Independence Ave., S.W. Washington, DC 20591	14. Sponsoring Agency Code

15. Supplemental Notes

Work was accomplished under approved task AM-B-96-TOX-202

16. Abstract

Introduction: One mission of the Civil Aeromedical Institute is to determine the concentrations of alcohol in postmortem specimens related to aviation accidents. This requires the ability to identify and quantitate a wide range of alcohols that are produced in postmortem specimens. A headspace gas chromatographic procedure utilizing n-propanol as an internal standard had been used in the past. However, n-propanol has been found in postmortem specimens, making n-propanol an unsuitable specimen for an internal standard in the analysis of postmortem specimens. This study evaluated 3 potential replacement internal standards for postmortem ethanol analysis. **Method**: A mixture of alcohols commonly found in postmortem specimens was prepared and tested using headspace gas chromatography. Solutions were prepared using the test mix and the new internal standards. Data were collected on the resolution and reproducibility of the proposed new internal standards with the test mix. Postmortem cases collected over the past 8 years were reviewed for the presence of specific volatile compounds. **Results**: Baseline resolution from the test mix was not obtained with propionaldehyde, while propionic acid methyl ester exhibited degradation over time. T-butanol was found to give baseline resolution from all volatile compounds commonly found in antimortem and postmortem specimens. No t-butanol was found in 2880 fatal pilots analyzed over the past 8 years for the presence of volatiles. **Conclusion:** t-butanol is a better internal standard for the analysis of alcohols in postmortem specimens than propionaldehyde, n-propanol, and propionic acid methyl ester, and is not produced in postmortem specimens.

17. Key Words	18. Distribution Statement
Alcohol, analysis, quantitation, aviation accidents	Document is available to the public through the National Technical Information Service, Springfield, Virginia 22161

19. Security Classif. (of this report)	20. Security Classif. (of this page)	21. No. of Pages	22. Price
Unclassified	Unclassified	10	

Form DOT F 1700.7 (8-72) Reproduction of completed page authorized

Selection of an Internal Standard For Postmortem Ethanol Analysis

INTRODUCTION

The Office of Aviation Medicine, Civil Aeromedical Institute (CAMI) is required under Public law 100-591 to help assess the role of potential medical or drug related pilot impairment in aviation accidents. This includes the identification and quantitation of various alcohols in addition to acetaldehyde and acetone found in postmortem specimens. The laboratory has tested 2880 specimens from fatal pilots over the past 8 years for the presence of alcohols (Table 1).

The CAMI Forensic Toxicology Laboratory has characteristically used n-propanol as an internal standard, using headspace gas chromatography analysis, for the identification and quantitation of various alcohols, acetaldehydes, and ketones that are typically found in postmortem specimens. However, various studies have reported the presence of n-propanol in some postmortem specimens containing ethanol.[9,13,15,18] According to Fumio Moriya, "n-propanol, which is produced with ethanol postmortem, can be an indicator of postmortem ethanol production because normally it does not exist in the living body".[18] This compound is the most commonly reported "other volatile associated with postmortem synthesis of ethanol."[16] The finding of n-propanol in postmortem specimens logically suggested a new internal standard be considered for ethanol analysis in postmortem specimens. The following criteria were emphasized in selecting an improved internal standard: (1) The chemical properties of the internal standard must be similar to the chemical properties of the compounds being quantitated or separated; (2) The retention time of the internal standard should be in the middle range of

Table 1. Positive alcohol cases for pilots involved in fatal accidents for the past 8 years.

Year	Fatal Pilots with 40mg/dL of ethanol or more	% of the Total Fatalities	Total Fatal Pilots
1989	28	8.0	349
1990	29	7.9	367
1991	30	7.7	389
1992	29	7.3	400
1993	30	8.8	340
1994	24	6.9	347
1995	15	4.3	352
1996	28	8.3	336
Total	213	7.4	2880

the retention times of the compounds being separated; (3) The internal standard must have baseline separation from all components of the mixture.

Considering these criteria, propionaldehyde, propionic acid methyl ester, and t-butanol were selected as potential replacements for the n-propanol internal standard. Propionaldehyde and propionic acid methyl ester were favorable choices because their retention times are close to the retention time of ethanol. The t-butanol was a favorable choice because it had alcohol chemical properties and a retention time in the middle range of those found in the test mix.

MATERIALS & METHODS

The gas chromatograph (GC) was an HP 5890 series II gas chromatograph with an FID detector, equipped with HP 19395A Headspace Sampler. The GC column was a 60/80 Carbopack B, 5% Carbowax 20, 6 foot X ¼-inch OD glass-packed column. The GC oven temperature was initially 65°C for 6.5 minutes, ramping at 20°C/min. to a final temperature of 140°C and held for 2 minutes at this temperature. The GC had an injection temperature of 150°C and a detector temperature of 170°C.

Preparation of "Working Test Mix"

The following test mix was prepared from a stock solution:

Volatile Component	Concentration (mg/dL)
Acetaldehyde	31.33 mg/dL
Methanol	158.28 mg/dL
Acetone	31.59 mg/dL
Ethanol	157.46 mg/dL
Isopropanol	157.10 mg/dL
n-Propanol	241.25 mg/dL
sec-Butanol	80.00 mg/dL
Isobutanol	32.76 mg/dL
n-Butanol	80.95 mg/dL

Preparation of Internal Standards

A 5.04 mg/dL solution of propionaldehyde internal standard was prepared. This reagent was stored at 2 to 8°C. A 17 mg/dL propionic acid methyl ester internal standard was prepared. This reagent was stored at 2 to 8°C. A 39.43 mg/dL t-butanol internal standard was prepared and stored at 2 to 8°C.

Preparation of Samples

Five hundred mL of the 17 mg/dL propionic acid methyl ester or t-butanol internal standard solution and 500 mL of the ethanol standard (150 mg/dL) or Working Test Mix were pipetted into an appropriately labeled 10 mL glass reaction vial. The vial was sealed immediately after the addition of the Working Test Mix or ethanol standard. This was repeated for all remaining vials. All specimens were vortexed to ensure all materials were well mixed. The propionaldehyde was rejected as an internal standard because it did not have baseline separation from compounds commonly found in postmortem specimens.

After calibration of the instrument with an internal standard, the analysis was run with known ethanol concentration of 150 mg/dL. This format was used for each run:

Well Position	Sample Type
Wells 1-3	Internal Standard & Working Test Mix
Wells 4-24	Internal Standard & 150 mg/dL Ethanol Standard

Analysis of Prior Year Case Reports

The results of 2880 cases, analyzed over the past 8 years, were examined for the presence of specific compounds detected in postmortem specimens, with particular emphasis on t-butanol.

RESULTS

Propionaldehyde had a retention time of 1.63 min. (Figure 1) and did not have baseline resolution from methanol and acetone. It eluted at the same retention time as an unidentified peak commonly seen in postmortem specimens. It had a short retention time relative to the compounds of interest.

Propionic acid methyl ester, with a retention time of 4.49 minutes, did resolve from the peaks of interest and had a retention time in the mid range of the compounds of interest (Figure 1). However, this internal standard was found to be unstable and

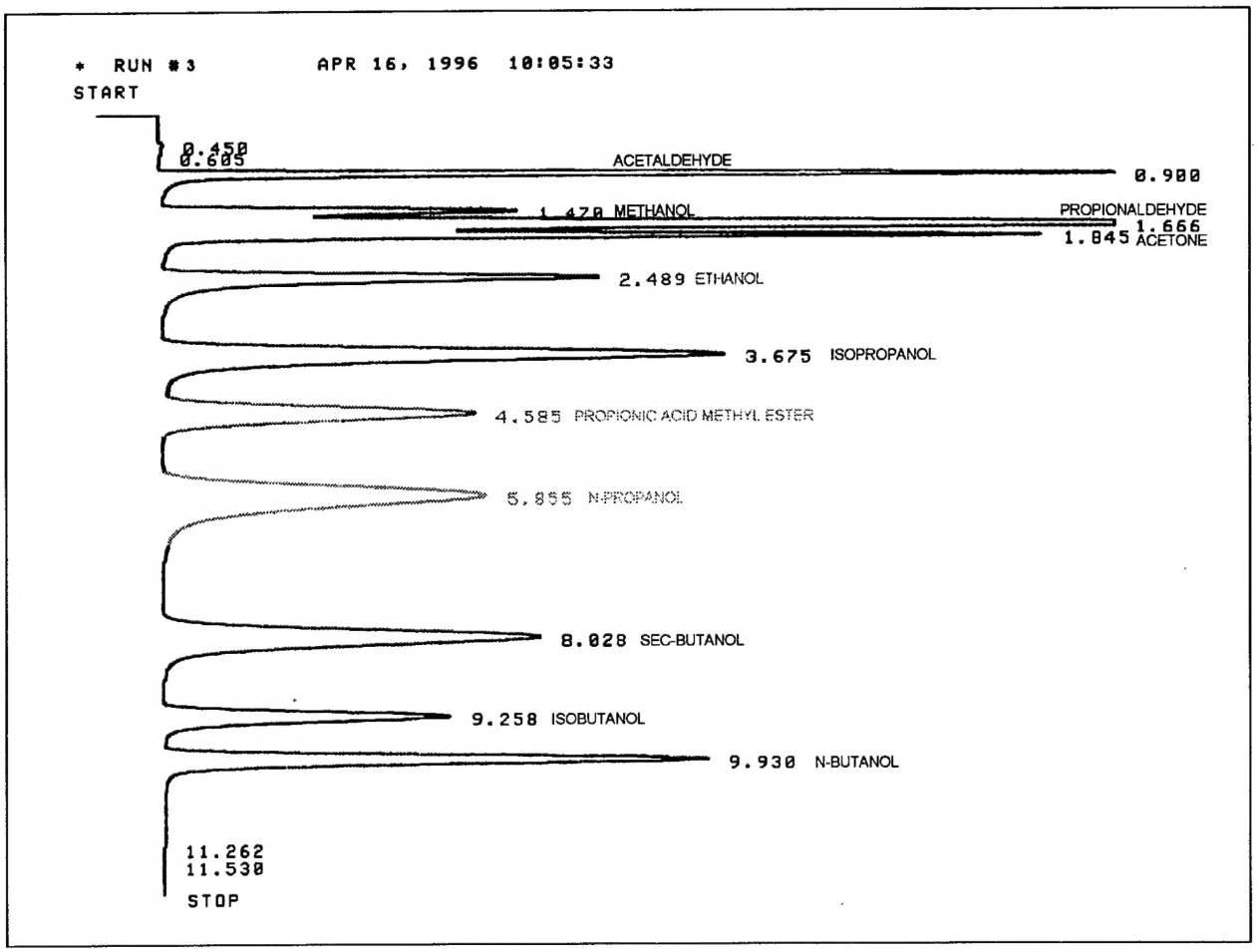

Figure 1. Gas Chromatograph of a standard test mix with 3 internal standards present. t-butanol was left out of test mix because it elutes at the same time as propionic acid methyl ester.

had a decreased peak area over time. This decrease in peak area for the internal standard resulted in what appeared to be an increasing concentration of ethanol with time for the same 150mg/dL control.

The t-butanol internal standard has a retention time of 4.86 minutes (Figure 2). It had acceptable baseline separation from the other components of the test mix. Twenty runs were made with a known concentration of ethanol of 150 mg/dL and the t-butanol (Table 2). The mean for the 150mg/dL concentration of ethanol was 149.75 mg/dL with a standard deviation of 2.24.

The n-propanol internal standard has a retention time of 5.73 minutes (Figure 2), which is not in the mid range of the retention times for the compounds of interest. N-propanol had acceptable baseline separation from the other components of the test mix. Twenty runs were made with a known concentration of ethanol of 150 mg/dL and the n-propanol (Table 2). The mean for the 150mg/dL concentration of ethanol was 149.9 mg/dL with a standard deviation of 3.48.

Although n-propanol has baseline separation from other compounds found in postmortem specimens, naturally occurring n-propanol found in postmortem specimens would interfere with the use of n-propanol as an internal standard. In contrast, no t-butanol was found in an examination of 2880 fatal pilots analyzed by the laboratory over the past 8 years (Table 1).

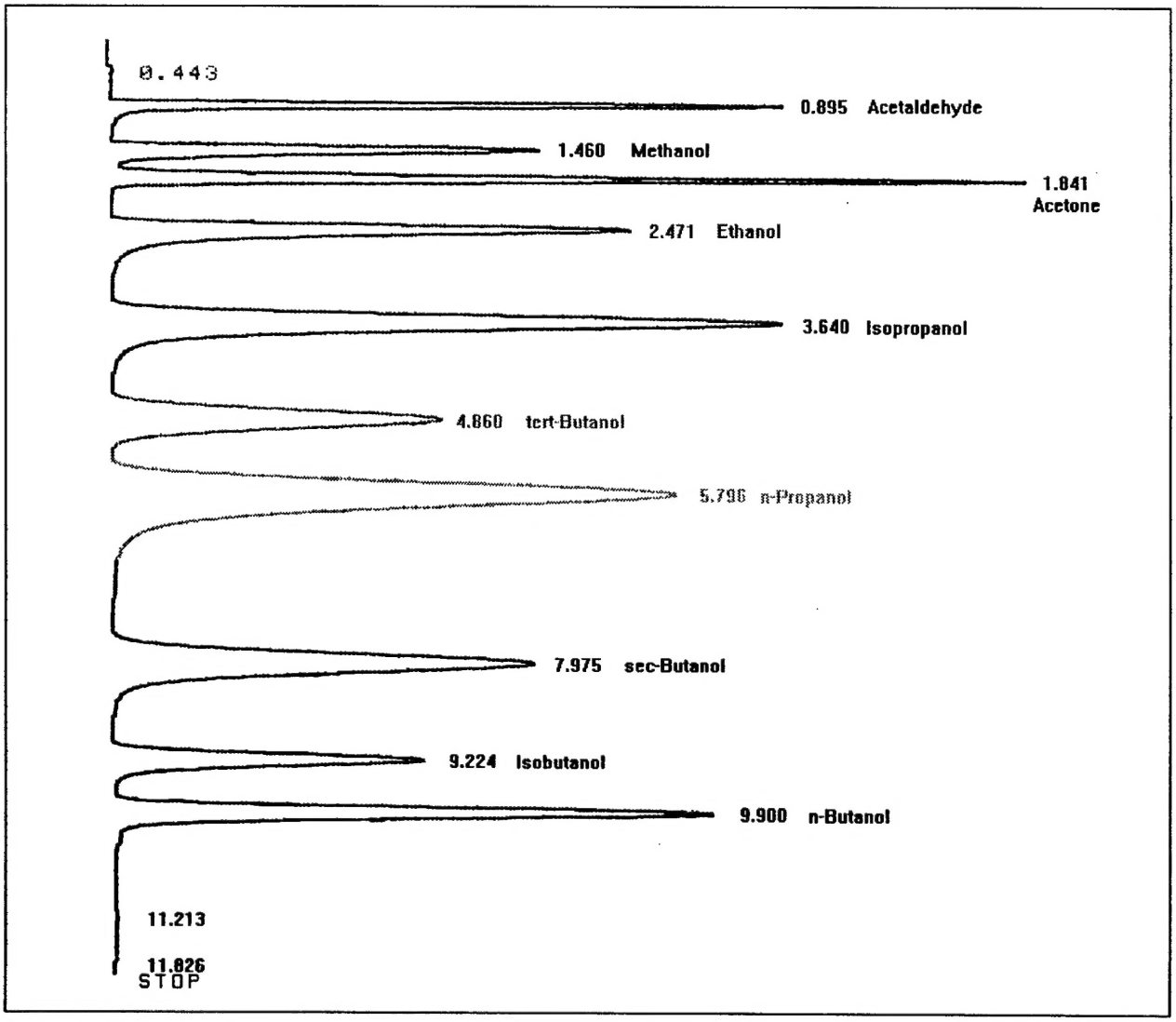

Figure 2. Gas Chromatograph of a standard test mix with t-butanol and n-propanol present.

Table 2. Comparisons of n-Propanol and t-Butanol internal standards.

Sample	n-Propanol	t-Butanol
1	144	146
2	150	146
3	152	148
4	150	147
5	152	147
6	149	151
7	147	151
8	148	153
9	156	152
10	150	151
11	149	152
12	154	150
13	154	151
14	153	151
15	145	150
16	148	150
17	148	148
18	146	147
19	156	152
20	147	152
SUM	2998	2995

n-Propanol	
Mean	149.9
SD	3.48
N	20
CV	2.32
VAR	12.09

t-Butanol	
Mean	149.75
SD	2.24
N	20
CV	1.50
VAR	5.04

DISCUSSION & CONCLUSION

One of the three candidates proposed as a new internal standard best met the criteria established for this study. Tert-butanol is not found in postmortem specimens, has a retention time and peak area similar to ethanol, has acceptable baseline separation from other components, and does not degrade with time. With t-butanol as an internal standard, the mean concentration for a 150 mg/dL ethanol standard was found to be 149.75 mg/dL, and the calculated standard deviation is 2.24. This is even better than the 3.48 standard deviation found for n-propanol.

Propionaldehyde was eliminated from consideration because it did not have acceptable baseline separation from compounds commonly found in postmortem samples. Propionic acid methyl ester was not found in postmortem specimens, had baseline separation, and had a retention time and peak area comparable to ethanol. Problems arose when the concentration of propionic acid methyl ester was found to decrease steadily with time. Propionic acid methyl ester is not a suitable internal standard for the analysis of ethanol because it degrades over time.

In summary, since t-butanol best met our selection criteria, t-butanol will be the future routine internal standard for use in the quantification of ethanol in postmortem specimens.

REFERENCES:

1. Alha AR, Tamminen V (1971). Detection of Alcohol in Aviation and Other Fatalities in Finland. *Aerospace Med, 42,* 564-8.
2. Briglia EJ, Bidanset JH, Dal Cortivo LA (1992). The Distribution of Ethanol in Postmortem Blood Samples. *J Forensic Sci, 37,* 991-8.
3. Blackmore DJ (1968). The Bacterial Production of Ethyl Alcohol. *J Forensic Sci Soc, 8,* 73-8.
4. Canfield DV, Kupiec TC, Huffine E (1993). Postmortem Alcohol Production in Fatal Aircraft Accidents. *J Forensic Sci, 38,* 914-7.
5. Caughlin JD (1994). An Unusual Source for Postmortem Findings of Methyl Ethyl Ketone and Methanol in Two Homicide Victims. *Forensic Sci Int, 67,* 27-31.
6. Chikasue F, Yashiki M, Miyazaki T, Okamotoa I, Kojima T (1988). Abnormally High Alcohol Concentration in the Heart. *Forensic Sci Int, 39,* 189-95.
7. Christmore D, Kelly RC, Doshier L (1984). Improved Recovery and Stability of Ethanol in Automated Headspace Analysis. *J Forensic Sci, 29,* 1038-44.
8. Davis G, Leffert R, Cpt. Rantanen N (1972). Putrefactive Ethanol Sources in Postmortem Tissues of Conventional and Germ-Free Mice. *Arch Pathol, 94,* 71-4.
9. Felby S, Nielsen E (1993). Postmortem Blood Alcohol Concentrations. *Blutalkohol, 30,* 244-50.
10. Gilliland MGF, Bost RO (1993). Alcohol in Decomposed Bodies: Postmortem Synthesis and Distribution. *J Forensic Sci, 38,* 1266-74.
11. Glendening B, Harvey R (1969). A Simple Method Using Head-Space Gas for Determination of Blood Alcohol by Gas Chromatography. *J Forensic Sci, 14,* 136-45.
12. Kuhlman JJ, Levine B, Smith ML, Hordinsky JR (1991). Toxicological Findings in Federal Aviation Administration General Aviation Accidents. *J Forensic Sci, 36,* 1121-8.
13. Moriya F, Ishizu H (1994). Can Microorganisms Produce Alcohol in Body Cavities of a Living Person?: A Case Report. *J Forensic Sci, 39,* 883-8.
14. Nanikawa R, Kotoku S (1974). Medicolegal Observations on a Dead Body Drawn Up From the Sea Bed, with Special Reference to Ethanol and Diatoms. *Forensic Sci, 3,* 225-32.
15. Nanikawa R, Ameno K, Hashimoto Y, Hamada K (1982). Medicolegal Studies on Alcohol Detected in Dead Bodies - Alcohol Levels in Skeletal Muscle. *Forensic Sci Int, 20,* 133-40.
16. O'Neal C, Poklis A (1996). Postmortem Production of Ethanol and Factors that Influence Interpretation. *Am J Forensic Med Pathol, 17,* 8-20.
17. Parker K, Fontan C, Yee J, Kirk P (1962). Gas Chromatographic Determination of Ethyl Alcohol in Blood for Medicolegal Purposes. *Anal Chem, 34,* 1234-6.

18. Takayasu T, Ohshima T, Tanaka N, Maeda H, Kondo T, Nishigami J, Nagano T (1995). Postmortem Degradation of Administered Ethanol-d6 and Production of Endogenous Ethanol: Experimental Studies Using Rats and Rabbits. *Forensic Sci Int, 76,* 129-40.

19. Winek T, Winek CL, Wahba WW (1996). The Effect of Storage at Various Temperatures on Blood Alcohol Concentration. *Forensic Sci Int, 78,* 179-85.

20. Zumwalt RE, Bost RO, Sunshine I (1982). Evaluation of Ethanol Concentrations in Decomposed Bodies. *J Forensic Sci, 27,* 549-54.

21. O'Neal, CL, Wolf, CE II, Levine, B, Kunsman, G, and Poklis, A (1996). Gas chromatographic procedures for the determination of ethanol in postmortem blood using t-butanol and methyl ethyl ketone as internal standards. *Forensic Sci Int, 83,* 31-8.

 ☆U.S. GOVERNMENT PRINTING OFFICE: 1998 -660-088/60058